Review article

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Microglial cells in neurodegenerative disorders

Marcin Wojtera[1], Beata Sikorska[2], Tomasz Sobow[1], Paweł P. Liberski[2]

[1]Department of Old Age Psychiatry and Psychotic Disorders, Medical University of Łódź; Poland; [2]Department of Molecular Pathology and Neuropathology, Medical University of Łódź, Poland

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Abstract

Microglia are resident immune cells of the CNS. They are involved in the pathogenesis of diverse neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, prion diseases as well as multiple sclerosis, amyotrophic lateral sclerosis and AIDS dementia complex. It is widely accepted that microglia contribute to the neurodegeneration through a release of a variety of proinflammatory substances. In fact, they are not the only cells which contribute to immunological processes inside the nervous system. The CNS is composed of different cell populations that answer to pathological factors and influence each other and modulate their reactions. These complex interactions are responsible for the development of brain pathology. This paper reviews the available information on microglial cells contribution to AD, PD and prion diseases development.

Key words: *microglia, neurodegeneration, Alzheimer's disease, prion disorders, Parkinson's disease.*

Introduction

Identification of a category of cells now known as microglia stemmed from the studies of del Rio-Hortega [23] and was based on metallic impregnation techniques. Microglia are resident immune cells of the central nervous system (CNS) which have the capacity to develop and proliferate into macrophages. The precise origin of microglial cells remains unclear. Studies published in the last three decades, however, usually supported the view that microglia are derived from cells of monocyte lineage that enter the CNS during the embryonic and early postnatal period of ontogenesis [18,55]. They assist in the remodeling and maturation of the brain and support clearance of cell remnants after apoptosis. In a mature normal brain, microglia are present as ramified cells having small cell bodies with numerous slender branching processes. They serve the role of immune surveillance and host defense. Microglia are very sensitive to changes in their microenvironment. In response to neuronal injury or infection ramified microglia transform into activated states – ameboid microglia [49]. Activated microglia up-regulate many surface receptors such as the major histocompatibility complex (MHC) or complement receptors and secrete a variety of soluble biologically active factors, which are either neurotrophic (e.g. Glia -Derived Neurotrophic Factor [GDNF]) or proinflammatory and neurotoxic (e.g. tumor necrosis factor alpha (TNFα), interleukin 1β (IL-1β), nitric oxide [NO] superoxide, eicosanoids, quinolinic acid) [7,67].

Communicating author:

Tomasz Sobow, PhD, Department of Old Age Psychiatry and Psychotic Disorders, Medical University of Łódź, 8/10 Czechosłowacka St., 92-216 Łódź, Poland, e-mail: tmsobow@csk.am.lodz.pl

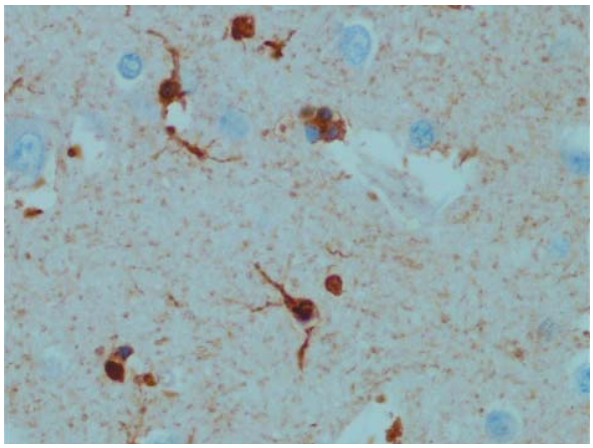

Fig. 1. Alzheimer's disease. Immunohistochemical reaction of microglial cells to antibody against ferritine, x400

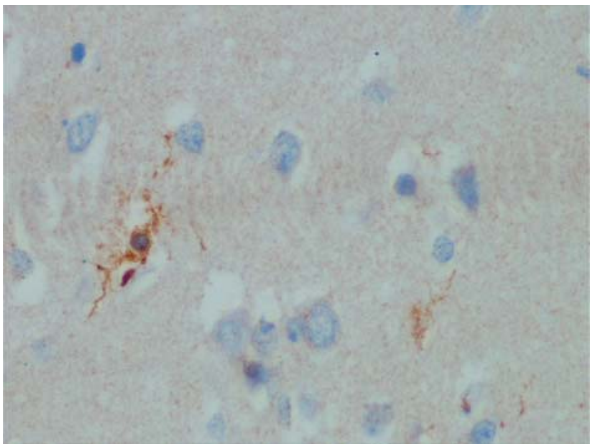

Fig. 2. Alzheimer's disease. Positive reaction to HLA DR on the surface of activated microglial cells, x400

Microglia are thought to be involved in the pathogenesis of diverse neurodegenerative diseases. Research in this area was inspired by neuropathological findings in Alzheimer's disease (AD) and Parkinson's disease (PD) brains. Reactive microglia were found to cluster frequently around the sites of amyloid deposition in the human brain, primarily at advanced neuritic plaques [42,45,83]. In brains from patients with Parkinson's disease (PD), microglia expressing MHC II receptors were found in the substantia nigra (SN), a key region of PD pathogenesis [61]. Since 1988, results of *in vivo* and *in vitro* studies have established an association of microglial activation in such diverse classes of diseases as prion diseases, multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS) and human immunodeficiency virus (HIV) acquired immunodeficiency syndrome dementia complex (ADC) [26, 28,56,81].

Nowadays, it is widely accepted that microglia contribute to the neurodegeneration through a release of variety of proinflammatory and potentially neurotoxic substances. It remains uncertain, however, what triggers activation of microglia in different disorders, if activated microglia means always the same: a destructive cell, how can we influence a disorder's natural prognosis by targeting the microglia?

Microglial markers

Del Rio-Hortega [23] visualized microglia using the silver carbonate impregnation method. With this method microglia appear as argentophilic cells but oligodendroglia, macrophages and capillaries are stained also. As a result, for many years scientists believed that microglia are merely monocytes invading injured brain tissues [48] and do not exist as a separate cell lineage.

The use of lectin histochemistry greatly enhanced the capability to identify microglia. RCA (Ricinnus communis agluttinin) and B4 isolectin from Griffonia simplicifolia (GSA I B4) are the main lectins used to this purpose. The immunocytochemical methods brought on a new era for research on microglia. Antibodies against ferritin, phosphotyrosine and keratan sulfate, glucose transported 5 (GLUT5) have been employed to recognize microglial cells. Studies using immunocytochemical markers have also confirmed that microglia are involved in immunological processes. Microglia have been shown to express antigens of the major histocompatibility complex (MHC) I and II as well as complement 3 receptor (CR3) and many others (for review see [41]) Unfortunately, a single, specific immunohistochemical marker for the microglial cell has yet to be described. The scanning electron microscopy (SEM) may provide a specific microglial marker as Giulian et al [36] showed that microglia from a postnatal rat brain are covered with spines (more than 20 per cell) in a distinctive manner [36], that contrasts with the smooth surfaces of bone marrow cells and the ruffled surfaces of tissue macrophages [40]. The spinebearing surface of microglia appears to be a specific cell marker, which is not changed with age or a variety of immunostimulants. However, the SEM is a

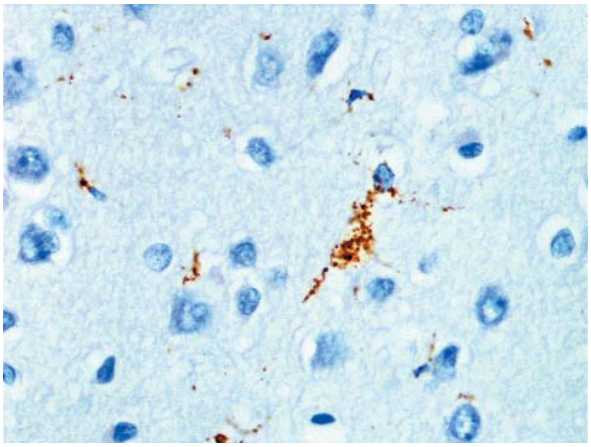

Fig. 3. Alzheimer's disease. Positive reaction of microglial cells to CD 68 antibody, x400

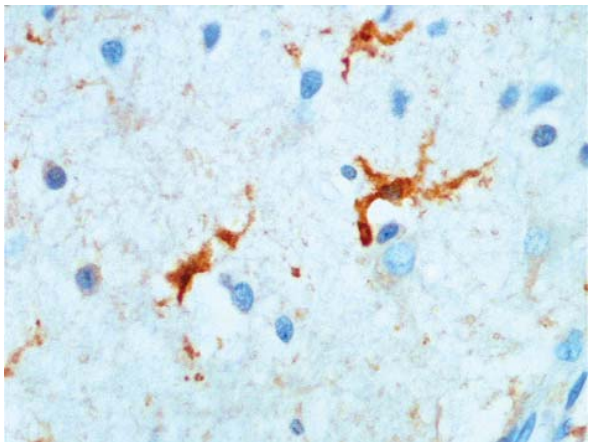

Fig. 4. CJD. Microglia, positive immunohistochemical reaction of ferritine antibody, x400

complicated method, which cannot be used in many *in vitro*, *ex vivo* and *in vivo* studies.

Microglia and AD

AD is a neurodegenerative disease characterized clinically by progressive cognitive decline and neuropathologically by a loss of neurons, primarily in the hippocampus and neocortical brain regions. Histopathological diagnosis of AD is based on the presence of Aβ-positive (amyloid β) plaques, and neurofibrillary tangles (NFTs).

The origin of amyloid plaques is attributed to extracellular deposition of β-amyloid (Aβ) which, in consequence, leads to a neuronal loss by an unclear mechanism, probably apoptosis and autophagy. According to the neuroinflammation theory of AD, the key pathomechanism of AD is "activation of the microglial cell". The neuroinflammation theory has originated from those studies that showed clustering of microglial cells within amyloid deposition in the human brains [42,45]. These studies have been reinforced by numerous publications showing immunological activity of microglia. Proinflammatory molecules such as cytokines, complement components and MHC II receptors were detected in the AD brain in association with microglia [21,39,82]. Studies on cultured microglia demonstrate that these cells can produce, in response to Aβ, a variety of neurotoxins (such as proteolytic enzymes, cytokines, complement proteins, reactive oxygen species, NMDA-like toxins, reactive nitrogen intermediates, TNFα) [35,69]. Early evidence from epidemiological studies supported the neuroinflammatory hypothesis, suggesting a beneficial effect of the prolonged use of nonsteroidal anti-inflammatory drugs (NSAIDs) in reducing the risk of developing AD (rev. [1]) Unfortunately, none of the prospective double blinded clinical trials have confirmed beneficial effects of NSAIDs. According to the neuroinflammatory hypothesis, microglia were cells which transform diffuse deposits of Aβ into compact senile plaques. Recently, an alternative way of plaque formation has been proposed, including models of cellular (neuronal, astroglial) and vascular origin.

Aβ deposits exist in many shapes and sizes, in fact they may reflect multiple mechanisms of plaque formation [20] and also differences in material processing (fixation and staining methods, post-mortem time delay etc.) [22]. The most frequent amyloid plaques are: dense-cored and diffuse plaques. It was suggested that diffuse and dense-cored plaques differ with respect to a glial activity. Senile plaques were primarily associated with highly active microglia [62,68]. In contrast, HLA – DR positive, activated microglia are not associated with diffuse plaque in neither human nor in transgenic APP23 mice [96,97].

The possibility that microglia may be involved in the formation of new amyloid plaques is very unlikely. Microglia express no detectable levels of βAPP mRNA; thus, they cannot synthesize Aβ from endogenous βAPP [87]. Moreover, Aβ deposition in brains with Down's syndrome (DS) is linked to an extra copy of

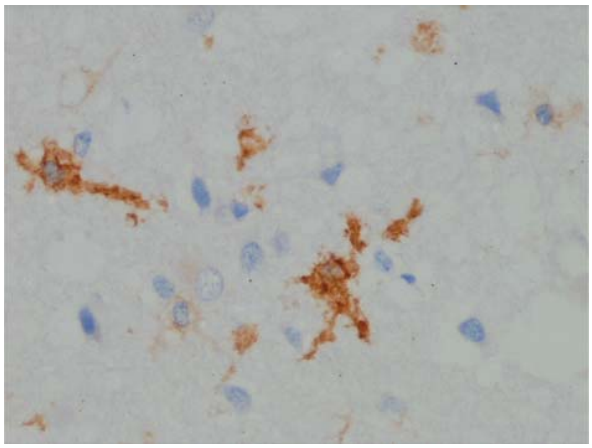

Fig. 5. CJD. Positive reaction of microglia to HLA DR antibody, x400

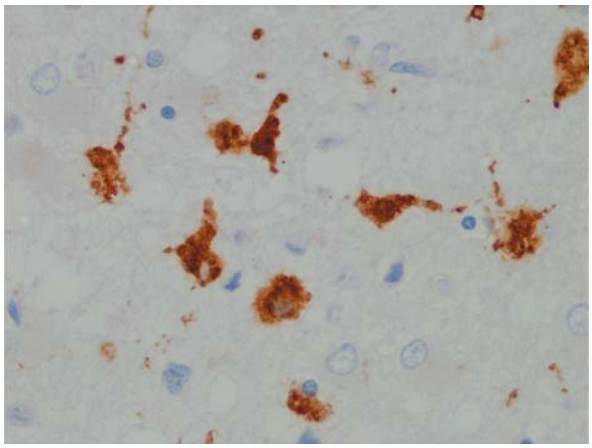

Fig. 6. CJD. Positive reaction of microglia to CD68 antibody, x400

βAPP gene and with no microglial involvement [53]. Furthermore, in the DS brain, activated microglia are associated only with merely particular types of amyloid plaques, similarly to AD [73].

There remains a question as to why microglia are clustered almost exclusively around dense-cored plaques but not in diffuse plaques (regarded by many but not all investigators as the first step to develop dense cored plaques).

A growing body of evidence suggests that every type of plaques represents a unique origin, contrary to an earlier hypothesis saying that they are merely sequential stages in the evolution of a single plaque type. In fact, many studies proved unsuccessful to support the hypothesis that diffuse plaques evolve into dense-cored plaques [3,58,93,97,108]; these may suggest that different plaques are not sequential phenomena but may have different origins [74,108]. Although a prevailing opinion is that amyloid plaques originate from extracellular deposition, plaques may also spring up from the vessels [70], neurons [19,21] Purkinje cell dendritic processes [99] or astrocytes [74]. Different ways of plaque formation may explain why some plaque types (dense-cored) are associated with microglial cells while others (diffuse) are mostly not. Beside Aβ, amyloid plaques contain many other substances such as lysosomal enzymes, cellular DNA, advanced glycation endproducts etc., which are known to be sufficient to activate microglia. In addition, Aβ aggregates without any cofactors are rather weak chemoattractants *in vitro* [69]. Because the main component of diffuse plaques – pure Aβ is a weak chemoattractant, it does not activate microglia. In contrast, dense-cored plaques are rich in cell-derived chemoattractants, which are sufficient to induce microglial activation and their migration to the center of the plaque.

Activation of microglia

One of the microglial activating materials found in senile plaques is the nuclear debris. Nucleotides are diffusible and may play a role in microglial chemotaxis through $G_{i/o}$-coupled P2Y receptors [27,44]. Neurons make complement components which opsonize Aβ. Opsonized Aβ is readily recognized and phagocytized by complement microglial receptors [88]. Microglial class A scavenger receptors, class B scavenger receptors B1, CD 36 [16,17,72,78], and Fc receptors [84] also participate in Aβ phagocytosis. Another strong microglial activator is a receptor for advanced glycation end-products (RAGE) and Aβ is known as a ligand for this receptor [105]. Finally, formyl peptide receptor-like 1 (FPRL1) is also involved in the proinflammatory response in AD [109]. It is responsible for activation, migration and polarization of microglial cells in response to Aβ.

In vitro studies

Findings from the *in vitro* studies have shown that cultured microglial cells may phagocyte fragments of amyloid stars isolated from a human brain [29] as well as Aβ [14,78,100]. Microglia actively phagocytose Aβ monomers, oligomers, and fibrils. However, the

data on fibrillar Aβ degradation in microglia are less consistent. Frackowiak [29] observed unmodified fibrillar amyloid in the cytoplasmic vacuoles up to the end of culture period [29]. Other studies showed a 10% reduction [8], partial degradation [14] and significant degradation [2] of Aβ in the presence of microglia *in vitro*.

Formation of fibrils was not seen in microglia cultures incubated with either monomeric or oligomeric Aβ 41-42. Clearance of Aβ monomer leads to the formation of oligomers (approximately 18kDa) visualized by the electron microscope in secondary lysosomes [2]. However, there are no conclusive data indicating that this process may lead to further Aβ fibrillisation.

It is interesting to note that fibrillar Aβ, the predominant Aβ species in dense cored plaques is associated with microglia only in the brain but not in congophylic angiopathy [93].

Animal models

In vivo studies showed that a direct injection of amyloid or amyloid fibrils into the rodent cerebral cortex results in Aβ phagocytosis, removal and formation of a glial scar [30,31,79]. Despite the presence of activated microglia, there were no neurofibrillary tangles. Therefore, this finding does not support the link between Aβ-activated microglia and the formation of NFTs [32]. However, it supports the notion that at least rodent microglia have the capability to remove Aβ.

In fact, many studies of microglia in human and transgenic mice amyloid plaques lend no support for a suggestion of Aβ internalisation and degradation [90,101-104]. To date, there is no ultrastructural documentation of Aβ phagocytosis by microglia within plaques or capillaries in humans, non-treated Tg mice and vaccinated mice. On the other hand, there are experiments with βAPP Tg mice in which microglia were stimulated by widely varying methods (entorhinal cortex lesion, passive and active Aβ vaccine, LPS injection, trauma, nitroflurbiprofen) and after that, the amyloid burden was reduced [6,12,25].

Furthermore, Bacskai et al [5] demonstrated a rapid microglial activation and plaque clearance in vivo using a multiphoton microscope [5].

Finally, postmortem histopathology carried out on humans who died after receiving Aβ vaccine showed fewer Aβ plaques in the neocortex rather than seen in nonimmunized AD patients suggesting that some Aβ clearance had occurred [75].

Vaccination trials are giving us evidence that diffuse plaques are more readily removed than dense-cored plaques. However, dense-cored plaques appear to be removable, too. Thus, activation of microglia does not lead to a plaque conversion from one type to another.

Moreover, experiments from Wyss-Coray et al [105] have shown that microglial activation is associated with lower amyloid load [105]. In this experiment with Tg mice, glial overexpression of TGFβ1 (thought to be major anti-inflammatory cytokine), markedly reduced plaques but increased microglial activation. In fact, TGFβ1 caused an increasing expression of complement C3 and promoted opsonization Aβ with C3b. Blocking C3 conversion and so Aβ opsonization prevents microglial amyloid clearance leading to a doubling of amyloid burden [106].

So why does such a discrepancy in results exist? On the one hand, there are *in vitro* experiments showing that microglia have the capacity to phagocyte and degrade amyloid, *in vivo* studies showing diminished amyloid load after promoting microglial activation. On the other hand, there is almost a lack of ultrastructural evidence that microglia *in vivo* are capable to phagocyte and digest amyloid. One possible explanation is that this may be the effect of missing elements such as a regulatory influence of astrocytes or senescence of microglia.

Astrocytes possess the potential to phagocyte and degrade amyloid. Aβ-overburdened astroglia decompose and form amyloid plaques [74]. Astrocytic activation is subsequent to the activation of microglia. Some of the in vitro studies indicated that the local presence of astrocytes inhibited the microglial ability to ingest plaques or Aβ [24,87]. Astrocytes cultured with Aβ released glycosaminoglycase-sensitive molecules that inhibited microglial Aβ removal. Furthermore, astrocytic-derived IL4 inhibited microglial activity *in vitro* [91]. Therefore, the activation of astrocytes may regulate the phagocytic microglial activity.

The other factor responsible for the inconsistence in AD microglial studies can be senescence and dysfunction of microglia (rev. [92]). According to this hypothesis old, dysfunctional microglia cannot provide enough protection to neurons and effectively phagocyte and degrade amyloid which leads to the evolution of hallmarks of AD pathology.

Microglia and PD

Parkinson's disease (PD) is a neurodegenerative disorder characterized by a progressive degeneration of dopaminergic neurons in the substantia nigra (SN). This region is responsible for movement control and its damage leads to the development of signs and symptoms: resting tremor, rigidity, bradykinesia, and gait disturbances. Over 95% PD cases are sporadic and of late onset [95]. Less than 5% of cases occur in familial clusters and have an early onset [71]. Several mutations have been identified (including genes encoding for parkin and α-synuclein) to be responsible for the development of familial PD. Idiopathic PD is the outcome of a complex interactions between genetic predisposition, and exposure to environmental factors, yet, however, to be defined (rev. [63]).

Dopaminergic neurons are particularly vulnerable to insults caused by a variety of factors. In fact, these neurons have a reduced antioxidant capacity due to a high content of dopamine, melanin and lipids. Thus, they are prone to oxidation and potential defects in the mitochondrial function [38,46]. Furthermore, microglia are particularly numerous in the SN area [52]. Therefore, sensitive dopaminergic neurons are residing in the potentially dangerous microglia – rich environment.

In 1988 McGeer and colleagues firstly observed, in the *post mortem* analysis, activation of microglia in the SN *pars compacta* and striatum of brains from patients with PD. An abundance of activated microglia is seen not only in idiopathic PD but also in familial PD [107]. Histopathological studies corroborating the neuroinflammation theory of PD are somehow supported by genetic studies. Genetic polymorphisms in genes encoding for proinflammatory cytokines such as IL1, IL-6, TNFα as well as for TNFα receptor are observed in PD patients [50,66,76,77,86].

Nevertheless, most of the changes seen in PD brains were detected in the terminal stage of the disease. A question remains whether activated microglia are the cause or merely a consequence of the neuronal loss. An answer to this question can be given in studies on animal models of PD.

Animal models of human PD

To support the hypothesis that inflammation may induce neurodegeneration in the nigrostriatal system, Castano [11] used bacterial LPS (Lipopolysaccharide) to evoke a neuroinflammation. LPS was injected directly into the SN area of rat brains causing quick, spectacular neurodegeneration. However, the degeneration was not selective to dopaminergic neurons and too fast to explain the temporal sequence of pathological events leading to PD [11]. A chronic LPS infusion model explains better a temporal relationship between microglial activation and degeneration of dopaminergic neurons [33]. In this model, LPS was chronically infused into the NS region of rat brains using an epidermal osmotic minipump. The highest microglial activation occurred in the first 2 weeks, but degeneration of dopaminergic neurons did not appear until 4-6 weeks after the LPS injection. In addition, the *in utero* exposure of rat foetuses to LPS caused degeneration of the nigrostriatal dopaminergic pathway in neonates [56]. Another microglia-activating substances, Fcγ receptor activators (trisialoganglioside, immunoglobulins from PD patients) also cause nigrostriatal degeneration of dopaminergic neurons [43,85].

Reactive microglia are seen in different animal models of PD such as 6-hydroxydopamine, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and rotenone models [15,64,89,94]. Reactive microglia are seen in the SN years after exposure to MPTP in monkeys [64] as well as in humans [51]. There are several studies the results of which have shown that anti-inflammatory drugs diminish dopaminergic toxicity in animal models of PD (for review see [65]).

The evidence that an inflammatory process is contributing to neurodegeneration in PD is growing. It seems plausible that anti-inflammatory treatment might slow down the progression of the disease. The animal studies are consistent with to date one prospective epidemiological study [13]. Participants of Chen's study who reported a regular use of nonaspirin NSAIDs at the beginning of the study had a lower risk of PD than nonregular users during the follow-up; the pooled multivariate relative risk was 0.55. Compared with nonusers, a nonsignificantly lower risk of PD was also observed among men and women who took 2 or more tablets of aspirin per day. This data is optimistic but needs to be confirmed.

Microglia and Prion diseases

Transmissible spongiform encephalopathies (TSEs) are fatal neurodegenerative and infectious disorders affecting humans (e.g. Creutzfeldt-Jakob disease-CJD,

kuru) and animals (e.g. sheep scrapie, bovine spongiform encephalopathy). Neuropathological characteristics for CJD are spongiform change, astrogliosis, neuronal loss, microglial activation and prion protein immunoreactivity. At the molecular level, TSEs are characterized by brain accumulation of a misfolded-protease resistant isoform (PrP^{sc}) of cellular prion protein (PrP^{c}) [80]. Conversion of PrP^{c} into PrP^{sc} results in a profound change in the biochemical properties of prion protein without any changes in amino acids sequence or posttranslational modifications. A neuronal loss in CJD is mainly caused by programmed cell death [34,37,47,54]. The time course of prion deposition, appearance of activated microglia, and death of neurons in *in vivo* models suggests that microglial activation precedes neuronal loss [98]. Activated microglia produce various soluble factors such as cytokines and free radicals that regulate neuronal and glial survival [110]. Microglia derived nitric oxide is thought to be responsible for neuronal programmed death [10]. Brown et al [9] have shown with an *in vitro* model (using synthetic prion fragment P106-126) that activated microglia are necessary to cause a neuronal loss [9]. Microglia might also play a role of a Trojan horse and spread the disease throughout the CNS [4].

It was not clear for many years what triggers the microglial recruitment to places of PrP^{sc} depositions. Nowadays there is a growing body of evidence that microglia recruitment occurs early during prion infection. Marella and Chabry [59] have shown that microglial recruitment occurs in vivo within few days after inoculation with PrP^{sc} positive material. This microglial recruitment is due to the response of neurons and astroglia to prion infection. PrP^{sc} stimulated neurons and astrocytes induced chemotactism by upregulation of chemokine expression [59]. The main two chemokines which mRNA was increased after PrP^{SC} stimulation were: RANTES (regulated on activation, normal T-cell expressed and secreted) and MIP-1β (macrophage inflammatory protein 1β). Both chemokines share a common receptor CCR-5. Using TAK-779 (CCR-5 antagonist) provoked a decrease of the microglial attraction rate in a dose-dependent manner. The authors have shown also that activated microglia, *via* secretion of soluble factors, can induce neuronal apoptosis. Neurons treated with microglial cell medium, precondotioned with hgtsc+ (homogenates of scrapie infected neuroblastoma cells), showed signs of apoptosis without any cell to cell interaction.

The intracellular mechanisms underlying the neuronal PrP^{sc} inducible expression of chemokines are unclear. Marella et al [60] showed that the mitogen-activated protein (MAP) kinase pathway in neurons is in part responsible for increased expression of RANTES after prion exposure [60].

Summary

Microglia contribute to many neuropathological processes. Microglial activation and neuroinflammation is responsible in part for neuronal dysfunction, injury, and loss (and hence to disease progression). Microglia are not the only cells, which play an important function in immunological processes inside the nervous system. The CNS is composed of different cell populations which influence each other. Different pathological factors influence neurons, astrocytes and microglia causing different cell reactions such as: production of proinflammatory/antiinflammatory cytokins or modulation of expression of different signalling peptides/proteins. Understanding these complex interactions may allow us to better treat neurodegenerative disorders.

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